

**STATISTICAL OBSERVATIONS ON THE TEXAS FEVER PARASITE.**

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The study herein described was based on material obtained from two cows, Nos. 1025 and 1031, of the series of the Experiment Station of the United States Bureau of Animal Industry. These, being so-called northern animals, were susceptible to Texas fever, and were both inoculated with this disease by infestation with infectious seed ticks on June 27, 1913. Cow No. 1031 died on July 10, and a series of preparations was obtained shortly after death from the heart muscle, the liver, the spleen, and the kidney. Cow No. 1025 died July 11, and a set of smears was obtained from the heart muscle.

The morphology of the Texas fever parasite, for which the correct name appears to be *Babesia bovis*, has been described a number of times and is in consequence a matter of common knowledge. This phase of the biology of the organism was therefore not studied, but since the material obtained, especially from Cow 1031, was rich in parasites and parasitized cells, a statistical investigation was undertaken. This had to do, first, with the relative numbers of parasitized and non-parasitized cells; second, with the number of individual parasites in each parasitized cell; third, with the varying conditions found in the several organs of Cow 1031; finally, a count was made of the parasites occurring free in the preparations. The exact significance of these latter is somewhat problematical, since, theoretically, they may either represent elements escaped from a blood cell, or merely what remains after the blood cell has been destroyed. - The latter supposition seems the more probable, since these parasites, like those within the cells, may occur either singly or in multiple form. Indeed, the fact that these apparent free parasites occur in multiple form rather suggests that they are still lying in some remnant of the blood cell which does not stain. The impression received from an examination of this material is that these "free" parasites have no especial significance, and are to be placed in the same category as those still lying in intact blood cells.

The smears were fixed in alcohol and stained with Giemsa. The method of study was very simple. A small square was cut on a

round cover glass with a diamond, and placed in the eye-piece of the microscope. The square marked out a small region in the smear and all the cells in this region were then taken into account. These regions were selected wholly at random, in fact, without being first seen. The only restriction was that those cases where the blood cells were very closely crowded were not used.

Taking up first the ratio of parasitized and non-parasitized cells, the counts made gave the following results:

TABLE 1.

Cow 1031	Parasitized cells	Non-parasitized cells	Total
Heart.....	4563	4083	8646
Kidney.....	767	464	1231
Spleen.....	348	2495	2843
Liver.....	1048	1646	2694
Totals	6726	8688	15414
Cow 1025			
Heart.....	250	3732	3982

Reducing the above to percentages we obtain:

TABLE 2.

Cow 1031	Parasitized	Non-parasitized
Heart.....	52.8 per cent.	47.2 per cent.
Kidney.....	62.3	37.7
Liver.....	38.9	61.1
Spleen.....	12.2	87.7
Totals	43.64	56.36
Cow 1025		
Heart.....	6.30	93.70

The first distinction to be noted is that between the two cows. In 1031, at least as regards the heart, the split is about even, there being practically as many parasitized as non-parasitized cells. On the other hand, in Cow 1025, the ratio is roughly 1 to 15. This cow survived for one day longer than did 1031, and this may be the explanation, but a much more precise study than was made would be necessary to elucidate this problem.

Turning our attention to Cow 1031, it is easily seen that there is a marked distinction between the conditions in the different organs. The heart, kidney, and liver may be grouped, although the differences between them are too great to be accredited to a mere accidental variation. It may also be noted that the liver shows a lower ratio of parasitized cells than does either the heart or kidney. This is in contrast to what occurs in infections with trypanosomes, these

parasites tending to persist in the liver after they have disappeared from other organs. At least this is stated to be the condition found post mortem.

But the very low percentage of parasitized cells present in the spleen, only 12.2 per cent, as against the general percentage of 43.64 in this cow is very clearly significant. It is usual to ascribe to the spleen a hemolytic function, and we appear to have an example of it here. The assumption is that the cells would themselves be destroyed, and along with them their contained parasites. In the case in point, however, it is necessary to assume that the parasitized cells are destroyed more easily than those which are still intact, but this is something which would be anticipated. The presence of the piroplasm in the cell would in all probability render it more susceptible to any destructive influence.

In the parasitized cells, the number of parasites per cell ranges from one to six, the following table showing the count:

TABLE 3.

	PARASITES PER CELL						Total.
	1.	2.	3.	4.	5.	6.	
Cow 1031							
Heart.....	3537	5354	247	151	4	1	9294
Kidney.....	1237	305	20	5	0	0	1567
Liver.....	2372	315	10	4	0	0	2701
Spleen.....	1301	85	2	1	0	0	1389
Cow 1025							
Heart.....	1317	1394	29	22	0	0	2762
Totals	9764	7458	308	183	4	1	17713

Reducing the above to percentages, we obtain:

TABLE 4.

	1.	2.	3.	4.	5.	6.
Cow 1031						
Heart.....	38.06	57.61	2.66	1.62	.04	.01
Kidney.....	78.94	19.46	1.28	.32	0	0
Liver.....	87.82	11.66	.37	.15	0	0
Spleen.....	93.67	6.12	.14	.07	0	0
Cow 1025						
Heart.....	47.68	50.47	1.05	.80	0	0
Totals	55.12	42.08	1.74	1.03	.02	.01

It is perhaps desirable to mention what at first glance looks like a discrepancy in the data. For example, in Table 3 there are given 2762 parasitized cells from the heart of Cow 1025, whereas in Table 1 this cow is credited with only 250 parasitized cells. This merely means that in the two cases the results were based on different counts. Thus, in the data given in Table 1 the cells were merely

separated into those that were parasitized and those that were not. It was found much easier to handle the material in this manner and the data given in Table 3 were based on a wholly separate set of observations from those given in Table 1. It is, however, believed that in obtaining the results set forth in Tables 1 and 2, the number of cells counted is large enough.

Turning our attention now to Tables 3 and 4, the percentages obtained from the slides taken from the hearts of Cows 1025 and 1031, respectively, are probably not far enough apart to warrant any conclusions, but a sharp distinction is to be seen between the findings in the different organs of Cow 1031. Thus, whereas, in the heart there were only some 38 per cent of the single parasites, this percentage rises to nearly 94 in the case of the spleen. It has already been suggested that the low percentage of parasitized cells in the spleen may be explained by supposing that the parasitized cell is more easily destroyed than that not parasitized. If this be so it would be consistent to suppose that as the number of parasites present increases, the invaded cell becomes more and more readily destroyed. For, since the parasite must enter the cell as a single organism, the multiplicative stages are indicative of a more prolonged occupancy, which in its turn is correlated with a greater damage to the cell. Moreover, two or more parasites occupy a greater volume than one, and this also would render the cell more liable to destruction. It is in support of this explanation that the liver stands next to the spleen both in regard to the proportion of parasitized cells and in the proportion of those which contain but a single parasite. These figures at least suggest that the liver is not a favorable environment for *Babesia*.

In addition to the above, a number of the so called "free" parasites were counted, the results being given in Tables 5 and 6. This could not be done with the spleen, which may be due either to the fact that the smears from this organ are never so clean as those from other situations, or to the fact that, as suggested, the parasites are here more quickly destroyed. The tables follow:

TABLE 5.

Cow 1031	1.	2.	3.	4.	5.	6.	Total
Heart.....	623	1057	38	39	0	0	1757
Kidney.....	1044	223	4	4	0	0	1275
Liver.....	262	28	0	0	0	0	290
Cow 1025							
	304	512	12	20	2	1	851
Totals	2233	1820	54	63	2	1	4173

Changing these figures to percentages we obtain:

TABLE 6.

Cow 1031						
Heart.....	35.46	60.16	2.16	2.22	0	0
Kidney.....	81.89	17.49	.31	.31	0	0
Liver.....	90.34	9.66	0	0	0	0
Cow 1025						
Heart.....	35.73	60.17	1.41	2.35	.23	.11
Totals	53.51	43.61	1.29	1.51	.05	.03

If Table 6 be compared with Table 4 it will be observed that there is a quite close accord so far as Cow 1031 is concerned. With regard to Cow 1025, however, there is a noticeable smaller percentage of the single forms. It is also to be noted that whereas in Table 3 there are a good many more parasites in the three stage than in the four, that this is reversed in Table 5.

Finally, as regards the number of parasites in each group, Tables 7 and 8 combine both those present in the cells and those which were apparently free.

TABLE 7.

	1.	2.	3.	4.	5.	6.	Total
Cow 1031							
Heart.....	4160	6411	285	190	4	1	11051
Kidney.....	2281	528	24	9	0	0	2842
Liver.....	2634	343	10	4	0	0	2991
Spleen.....	1301	85	2	1	0	0	1389
Cow 1025							
Heart.....	1621	1906	41	42	2	1	3613
Totals	11997	9273	362	246	6	2	21886

Reducing to the usual percentages we get:

TABLE 8.

	1.	2.	3.	4.	5.	6.
Cow 1031						
Heart.....	37.64	58.01	2.58	1.72	.04	.01
Kidney.....	80.26	18.58	.84	.32	0	0
Liver.....	88.07	11.47	.33	.13	0	0
Spleen.....	93.67	6.12	.14	.07	0	0
Cow 1025						
Heart.....	44.87	52.75	1.13	1.16	.06	.03
Totals.....	54.82	42.37	1.65	1.12	.03	.01

The percentages of Table 8 run very close to those of both Tables 4 and 6, which was of course to be expected. It may be of interest to note that of nearly twenty-two thousand cases there were only six groups of five and two groups of six.

With regard to the shape of the paired parasites, they were either approximately round or approximately piriform. Of 741 cases noted, in 406 the parasites were round, in 335 piriform. It may be mentioned, however, that the parasites found in smears of the organs made post-mortem are always smaller and rounder than those in smears made from the peripheral blood of the living cow. To the best of my knowledge, the only author who has emphasized this fact is Theobald Smith.